

法国产夏枯草中的两个新的乌索烷型三萜皂甙

张颖君 杨崇仁*

(中国科学院昆明植物研究所植物化学开放研究实验室, 昆明 650204)

摘要 从法国产夏枯草 (*Prunella vulgaris* L.) 的甲醇提取物中分离得到两个新的乌索烷型三萜皂甙: 夏枯草甙 (pruvuloside) A 和 B; 同时还分离到 5 个已知化合物: niga-ichigoside F_2 , sericoside, 槲皮素 (quercetin), 槲皮素-3-O-葡萄糖甙 (quercetin 3-O-glucoside), 山奈酚-3-O-葡萄糖甙 (kaempferol-3-O-glucoside), 及 arjunglucoside I 和 niga-ichigoside F_1 的混合物。它们的结构是通过波谱的方法证明的。

关键词 唇形科, 夏枯草, 三萜皂甙, 夏枯草甙 A, B

TWO NEW URSANE GLYCOSIDES FROM PRUNELLA VULGARIS IN FRANCE

ZHANG Ying-Jun, YANG Chong-Ren*

(Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204)

Abstract Two new ursane glucosides, pruvuloside A and B, together with five known compounds, niga-ichigoside F_2 , sericoside, quercetin, quercetin-3-glucoside, kaempferol-3-O-glucoside, and a mixture of niga-ichigoside F_1 and arjunglucoside I were isolated from the MeOH extract of *Prunella vulgaris* collected in France. Their structures were determined by means of spectral evidence.

Key words Labiatae, *Prunella vulgaris*, Triterpenoid saponins, Pruvuloside A, B.

Prunella vulgaris L. (Labiatae) as a perennial herb is widely distributed in the temperate zone and tropical mountain area of the whole world. As a wide-disperse species, this plant has been divided as several subspecies and varieties in different places according to their different physiological and ecological characters. It has been used as traditional Chinese medicine for treatment of tuberculosis, pleuritis, bacterial dysentery, icterohepatitis^[1]. In Japan only its spike in the flowering period is used as diuretic, and in Europe the whole herb has been used as the same use^[2]. Up to now, there were a lot of chemical studies on this plant, and some sterols, β -sitosterol, stigmasterol, stigmast-7-en-3 β -ol, α -spinasterol^[2-3]; triterpenoids, ursolic acid, oleanolic acid, 2 α , 3 α , 24-trihydroxyolean-12-en-28-oic acid, methyl 2 α , 3 α , 23-trihydroxyolean-12-en-28-oate^[2], methyl (13S, 14R)-2 α , 3 α , 24-trihydroxyolean-11-en-28-oate^[4], 2 α , 3 α , 24-trihydroxyolean-11, 13(18)-dien-28-oic acid^[5], et al., were isolated. This paper deal with the isolation and structure elucidation of two new ursane glucosides, pruvuloside A (I)

*通信联系人 Author to whom correspondence should be addressed.

and **B (2)**, together with two known saponins, niga-ichigoside **F₂(3)**, sericoside **(4)**, three known flavonoids, quercetin **(5)**, quercetin-3-O-glucoside **(6)**, kaempferol-3-O-glucoside **(7)** and a mixture **(8)** of niga-ichigoside **F₁(8a)** and arjunglucoside **I (8b)** from this plant.

RESULTS AND DISCUSSION

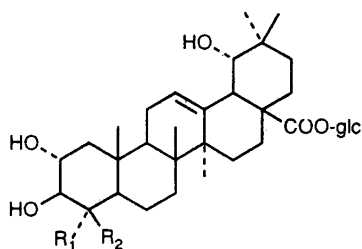
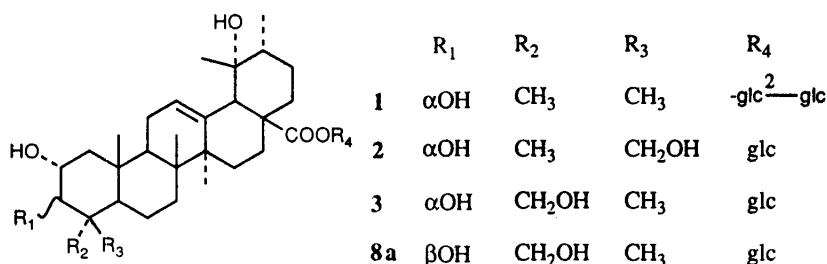
MeOH extract of *P. vulgaris* was suspended in H₂O and then successively extracted with Et₂O, AcOEt and n-Butanol. The n-Butanol extract was repeatedly chromatographed on Sephadex, Rp-8 and silica gel column to give six compounds, 1—7 and a mixture **(8)**. By means of NMR, compounds 3—4 were identified as known β -glucosyl ester of A-ring oxygenated 19 α -hydroxyursolic and oleanolic acid, niga-ichigoside **F₂(3)** ^[6] and sericoside **(4)** ^[7]; compounds 5—7 were identified as the known flavonoids, quercetin **(5)**, quercetin-3-O-glucoside **(6)**, kaempferol-3-O-glucoside **(7)** ^[8], and the mixture **(8)** was determined as niga-ichigoside **F (8a)** ^[6] and arjunglucoside **I (8b)** ^[9].

Compound 1 showed a quasi molecular ion peak at m/z 811 [$M(C_{42}H_{68}O_{15})-H$]⁻ and typical fragment ion at m/z 649 [$M-glc$]⁻, 487 [$M-glc-glc$]⁻ in the negative FAB mass spectrum. The ¹H and ¹³C NMR signals of **1** due to sugar moiety, indicated the presence of two anomeric signals. The ¹³C signals of **1** in pyridine-d₅ [δ 177.0 (CO₂- β Glc) and 93.8 (anomeric C of β Glc)], indicated the location of a β -glucopyranosyl group of **1** on COOH group of C-28. Comparison of the ¹³C NMR data of **1** with that of kaji-ichigoside **F₁** ^[6], showed that their carbon signals for the aglycone moiety were almost superimposable except for sugar moiety. **1** has one more glucose than kaji-ichigoside **F₁**, and from **1** to kaji-ichigoside **F₁**, the carbon signals of glucose are shifted by +2.2 (C-1'), -5.6 (C-2'). So, the terminal glucose of **1** was attached at C-2 position of the inner glucose which linked at COOH group of C-28, and this sugar chain were also supported by the comparison of the ¹³C NMR data with that of β -sophorosyl ester of hederagenin ^[10]. Thus, the structure of this new triterpenoidal saponin was established as 2 α , 3 α , 19 α -trihydroxyurs-12-en-28-oic acid-28-O- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside, and named as pruvuloside A.

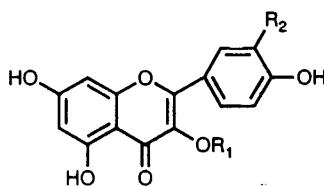
Compound 2 showed a quasi molecular ion peak at m/z 665 [$M(C_{36}H_{58}O_{11})-H$]⁻ and typical fragment ion at m/z 503 [$M-glc$]⁻ in the negative FAB mass spectrum. The ¹H and ¹³C NMR signals of **2** due to sugar moiety, indicated the presence of one anomeric signals. The ¹³C signals of **2** in pyridine-d₅ [δ 177.0 (CO₂- β Glc) and 95.9 (anomeric C of β Glc)] indicated the location of a β -glucopyranosyl group of **2** on COOH group of C-28. Comparison of the ¹³C NMR data of **2** with that of niga-ichigoside **F₂** ^[6], showed that all of their carbon signals were almost superimposable except for the signals of C-23 and C-24. Usually, the chemical shift of the C-23 methyl group is at about δ 24.4 if the hydroxyl group is linked at C-24 (δ 65.7) ^[9] and the chemical shift of C-24 methyl group is at about δ 16.7 if the hydroxyl group is linked at C-23 (δ 71.3) ^[6]. The chemical shifts of C-23 (δ 23.7) and C-24 (δ 65.3) in compound **2** suggested that the hydroxyl group was linked at C-24. Therefore, the structure of **2** was established as 2 α , 3 α , 19 α , 24-tetrahydroxyurs-12-en-28-oic acid-28-O- β -D-glucopyranoside. This new triterpenoidal saponin named as pruvuloside B.

This is the first report for the isolation of triterpenoid saponins from *P. vulgaris*. Among these compounds we obtained, **6** is the main compound in this plant, and all of the saponins have a hydroxy group at C-19 position and sugar moiety linked at the ester of C-28 of the triterpenoid skeleton, which is different

with the previous reports about this plant.



	R ₁	R ₂
4	CH ₃	CH ₂ OH
8b	CH ₂ OH	CH ₃



	R ₁	R ₂
5	H	OH
6	glc	OH
7	glc	H

EXPERIMENT

¹H and ¹³C NMR spectra were measured on a Bruker spectropin AM-400 spectrometer using TMS as internal standards. FAB-MS spectra were measured with VG Autospec mass spectrometer.

Extraction and isolation MeOH extract of *Prunella vulgaris* (46.69 g) was suspended in H₂O and then successively extracted with Et₂O, AcOEt and n-Butanol. The n-Butanol layer was concentrated in vacuo to give a viscous residue (5.09 g), which was repeatedly chromatographed on Sephadex (eluting with 80% MeOH), Rp-8 (eluting with 50% MeOH) and silica gel column (eluting with CHCl₃-MeOH-H₂O, 60 : 10 : 1) to give **1** (6 mg), **2** (5 mg), **3** (11 mg), **4** (10 mg), **5** (15 mg), **6** (75 mg), **7** (22 mg) and a mixture (**8**) (25 mg).

Pruvuloside A (1). a white powder. $[\alpha]_D^{24}$ -2.6° (MeOH, c 0.39); FAB-MS: m/z 811 [M(C₄₂H₆₈O₁₅)-H]⁺, 649 [M-glc]⁺, 487 [M-glc-glc]⁺; ¹H NMR (C₅D₅N): δ 2.70 (1H, s, H-18), 1.61, 1.34, 1.18, 1.11, 0.95, 0.82 (each 3H, s, H-23, 24, 25, 26, 27, 29 respectively), 1.05 (3H, d, J = 6.5 Hz, H-30), 5.70 (1H, br s, H-12), 6.22 (1H, d, J = 8.2 Hz, inner H_{glu}-1'), 5.70 (1H, d, J = 7.8 Hz, terminal H_{glu}-1'); ¹³C NMR (C₅D₅N): δ 43.0 (C-1), 66.2 (C-2), 79.1 (C-3), 38.7 (C-4), 48.7 (C-5), 18.7 (C-6), 33.7 (C-7), 40.9 (C-8), 47.8 (C-9), 38.8 (C-10), 24.2 (C-11), 128.4 (C-12), 139.5 (C-13), 42.2 (C-14), 29.8 (C-15), 26.0 (C-16), 48.9 (C-17), 54.6 (C-18), 72.8 (C-19), 42.2 (C-20), 26.8 (C-21), 37.9 (C-22), 29.4 (C-23), 22.3 (C-24), 16.9 (C-25), 17.5 (C-26), 24.7 (C-27), 177.0 (C-28), 26.1 (C-29), 16.8 (C-30), inner glucose: 93.8 (C-1'), 79.4 (C-2'), 78.1 (C-3'), 71.0 (C-4'), 79.1 (C-5'), 62.4 (C-6'), terminal glucose: 104.9 (C-1''), 76.0 (C-2''), 78.1 (C-3''), 72.9 (C-4''), 78.4 (C-5''), 63.9 (C-6'').

Pruvuloside B (2). A white powder. $[\alpha]_D^{24}$ -5.1° (MeOH, c 0.20); FAB-MS: m/z 665 [M

($C_{36}H_{58}O_{11}$)-H], 503 [M-glc]⁻; ¹H NMR (C_5D_5N): δ 2.90 (1H, s, H-18), 1.65, 1.61, 1.32, 1.18, 0.92 (each 3H, s, H-23, 25, 26, 27, 29 respectively), 1.05 (3H, d, J=6.6 Hz, H-30), 6.27 (1H, d, J=8.0 Hz, $H_{glu-1'}$); ¹³C NMR (C_5D_5N): δ 43.3 (C-1), 66.3 (C-2), 79.0 (C-3), 42.2 (C-4), 44.2 (C-5), 19.2 (C-6), 33.3 (C-7), 40.9 (C-8), 48.0 (C-9), 38.7 (C-10), 24.5 (C-11), 128.5 (C-12), 139.2 (C-13), 40.9 (C-14), 29.9 (C-15), 26.8 (C-16), 48.7 (C-17), 54.5 (C-18), 72.8 (C-19), 42.2 (C-20), 27.1 (C-21), 37.7 (C-22), 23.7 (C-23), 65.3 (C-24), 17.7 (C-25), 17.2 (C-26), 24.5 (C-27), 177.0 (C-28), 27.1 (C-29), 16.7 (C-30), 95.9 ($C_{glu-1'}$), 74.1 (C-2'), 79.0 (C-3'), 71.5 (C-4'), 79.2 (C-5'), 62.6 (C-6').

Niga-ichigoside F₂(3). a white powder. $[\alpha]_D^{24}+4.0^\circ$ (MeOH, c 0.75); FAB-MS: m/z 665 [M($C_{36}H_{58}O_{11}$)-H]⁻, 503 [M-glc]⁻; ¹H NMR (C_5D_5N): δ 3.72, 3.89 (each 1H, d, J=10.8 Hz, H-23), 2.70 (1H, s, H-18), 1.62, 1.42, 1.32, 1.22, 0.85 (each 3H, s, H-24, 25, 26, 27, 29 respectively), 1.04 (3H, d, J=8.2 Hz, H-30), 6.30 (1H, d, J=8.1 Hz, $H_{glu-1'}$); ¹³C NMR (C_5D_5N): δ 42.9 (C-1), 66.3 (C-2), 79.0 (C-3), 42.3 (C-4), 43.6 (C-5), 18.6 (C-6), 33.3 (C-7), 40.8 (C-8), 47.8 (C-9), 38.5 (C-10), 24.2 (C-11), 128.5 (C-12), 139.4 (C-13), 42.0 (C-14), 29.2 (C-15), 26.2 (C-16), 48.7 (C-17), 54.5 (C-18), 72.7 (C-19), 42.2 (C-20), 26.8 (C-21), 37.8 (C-22), 71.3 (C-23), 16.7 (C-24), 17.8 (C-25), 17.2 (C-26), 24.6 (C-27), 177.0 (C-28), 27.1 (C-29), 16.7 (C-30), 95.9 ($C_{glu-1'}$), 74.1 (C-2'), 79.0 (C-3'), 71.3 (C-4'), 79.3 (C-5'), 62.5 (C-6').

Sericoside (4). a white powder $[\alpha]_D^{24}+4.3^\circ$ (MeOH, c 0.24); FAB-MS: m/z 665 [M($C_{36}H_{58}O_{11}$)-H]⁻, 503 [M-glc]⁻; ¹H NMR (C_5D_5N): δ 3.55 (1H, br s, H-18), 1.56, 1.42, 1.24, 1.13, 0.96, 0.89, (each 3H, s, H-23, 25, 26, 27, 29, 30), 6.37 (1H, d, J=8.0 Hz, $H_{glu-1'}$); ¹³C NMR (C_5D_5N): δ 47.6 (C-1), 68.8 (C-2), 85.9 (C-3), 44.0 (C-4), 56.7 (C-5), 19.6 (C-6), 33.7 (C-7), 40.4 (C-8), 48.6 (C-9), 38.6 (C-10), 24.6 (C-11), 123.3 (C-12), 144.4 (C-13), 42.2 (C-14), 29.1 (C-15), 28.1 (C-16), 46.6 (C-17), 44.8 (C-18), 81.2 (C-19), 35.6 (C-20), 28.7 (C-21), 33.1 (C-22), 24.2 (C-23), 65.7 (C-24), 17.6 (C-25), 17.3 (C-26), 24.8 (C-27), 177.3 (C-28), 28.7 (C-29), 24.6 (C-30), 95.9 ($C_{glu-1'}$), 74.2 (C-2'), 79.0 (C-3'), 71.3 (C-4'), 79.2 (C-5'), 62.4 (C-6').

Quercetin (5). a yellow powder. FAB-MS: m/z 301 [M($C_{15}H_{10}O_7$)-H]⁻; ¹H NMR (DMSO- d_6): δ 7.66 (1H, s, H-2'), 7.53 (1H, d, J=8.4 Hz, H-5'), 6.87 (1H, d, J=8.4 Hz, H-6'), 6.45 (1H, s, H-6), 6.17 (1H, s, H-8); ¹³C NMR (DMSO- d_6): 146.8 (C-2), 135.7 (C-3), 175.8 (C-4), 156.1 (C-5), 98.2 (C-6), 164.0 (C-7), 93.4 (C-8), 160.7 (C-9), 102.9 (C-10), 121.9 (C-1'), 115.6 (C-2'), 145.0 (C-3'), 147.7 (C-4'), 115.0 (C-5'), 119.9 (C-6').

Quercetin-3-O-glucoside (6). A yellow powder. FAB-MS: m/z 463 [M($C_{21}H_{20}O_{12}$)-H]⁻, 300 [M-glc-H]⁻; ¹H NMR (CD₃OD+DMSO- d_6): δ 7.72 (1H, s, H-2'), 7.59 (1H, d, J=8.4 Hz, H-5'), 6.88 (1H, d, J=8.4 Hz, H-6'), 6.38 (1H, s, H-6), 6.18 (1H, s, H-8), 5.34 (1H, d, J=7.3 Hz, $H_{glu-1''}$); ¹³C NMR (CD₃OD+DMSO- d_6): 158.3 (C-2), 135.6 (C-3), 179.3 (C-4), 162.9 (C-5), 99.9 (C-6), 165.8 (C-7), 94.8 (C-8), 158.8 (C-9), 105.7 (C-10), 123.1 (C-1'), 116.1 (C-2'), 145.9 (C-3'), 149.8 (C-4'), 117.7 (C-5'), 123.1 (C-6'), 104.2 (C-1''), 75.7 (C-2''), 78.1 (C-3''), 71.3 (C-4''), 78.4 (C-5''), 62.6 (C-6'').

Kaempferol 3-O-glucoside (7). A yellow powder. FAB-MS: m/z 447 [M($C_{21}H_{20}O_{11}$)-H]⁻; ¹H NMR (DMSO- d_6): δ 8.02 (2H, d, J=8.8 Hz, H-2', 6'), 6.87 (2H, d, J=8.3 Hz, H-3', 5'), 6.41 (1H, s, H-8), 6.18 (1H, s, H-6), 5.43 (1H, d, J=7.4 Hz, $H_{glu-1''}$); ¹³C NMR (DMSO- d_6): 156.5 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.9 (C-6), 164.8 (C-7), 93.7 (C-8), 156.3 (C-9), 103.8 (C-10), 120.9 (C-1'), 130.8 (C-2'), 115.1 (C-3'), 160.0 (C-4'), 115.1 (C-5'), 130.8 (C-6'), 101.1 (C-1''), 74.3 (C-2'').

76.5 (C-3''), 70.0 (C-4''), 77.4 (C-5''), 60.9 (C-6'').

Mixture (8): a white powder.

Niga-ichigoside F₁ (8a). FAB-MS: m/z 665 $[M(C_{36}H_{58}O_{11})-H]^-$, 503 $[M-glc]^-$; 1H NMR (C_5D_5N): δ 2.71 (1H, s, H-18), 6.30 (1H, d, $J=8.1$ Hz, $H_{glu}-1'$); ^{13}C NMR (C_5D_5N): δ 48.0 (C-1), 69.0 (C-2), 78.6 (C-3), 44.7 (C-4), 48.5 (C-5), 18.8 (C-6), 33.3 (C-7), 40.8 (C-8), 48.2 (C-9), 38.5 (C-10), 24.3 (C-11), 128.5 (C-12), 139.3 (C-13), 42.3 (C-14), 29.3 (C-15), 26.2 (C-16), 48.7 (C-17), 54.5 (C-18), 72.8 (C-19), 42.2 (C-20), 26.8 (C-21), 37.7 (C-22), 67.0 (C-23), 14.3 (C-24), 17.6 (C-25), 17.5 (C-26), 24.6 (C-27), 177.0 (C-28), 27.1 (C-29), 16.7 (C-30), 95.9 ($C_{glu}-1'$), 74.1 (C-2'), 79.0 (C-3'), 71.3 (C-4'), 79.2 (C-5'), 62.4 (C-6').

Arjunglucoside I (8b). FAB-MS: m/s 665 $[M(C_{36}H_{58}O_{11})-H]^-$, 503 $[M-glc]^-$; 1H NMR (C_5D_5N): δ 2.90 (1H, br s, H-18), 6.38 (1H, d, $J=8.1$ Hz, $H_{glu}-1'$); ^{13}C NMR (C_5D_5N): δ 48.0 (C-1), 69.0 (C-2), 78.6 (C-3), 44.7 (C-4), 48.5 (C-5), 18.8 (C-6), 33.3 (C-7), 40.4 (C-8), 48.2 (C-9), 38.7 (C-10), 24.3 (C-11), 123.8 (C-12), 144.4 (C-13), 42.3 (C-14), 29.3 (C-15), 29.1 (C-16), 46.6 (C-17), 43.6 (C-18), 81.2 (C-19), 35.6 (C-20), 29.1 (C-21), 33.1 (C-22), 67.0 (C-23), 14.2 (C-24), 17.8 (C-25), 17.4 (C-26), 24.9 (C-27), 177.3 (C-28), 28.7 (C-29), 24.8 (C-30), 95.9 ($C_{glu}-1'$), 74.2 (C-2'), 79.0 (C-3'), 71.5 (C-4'), 79.2 (C-5'), 62.6 (C-6').

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References

- [1] Jiangsu New Medical College. The Dictionary of Traditional Chinese Medicine. Shanghai: Shanghai People press, 1977. 1827.
- [2] Kojima H, Ogura H. Triterpenoids from *Prunella vulgaris*. *Phytochemistry*, 1986, **25**(3): 729—733.
- [3] Kojima H, Sato N, Hatano A, *et al.* Sterol glucosides from *Prunella vulgaris*. *Phytochemistry*, 1990, **29**(7): 2351.
- [4] Kojima H, Tominaga H, Sato S, *et al.* Two novel hexacyclic triterpenoids from *Prunella vulgaris*. *Phytochemistry*, 1988, **27**(9): 2921—2925.
- [5] Kojima H, Tominaga H, Sato S, *et al.* Pentacyclic triterpenoids from *Prunella vulgaris*. *Phytochemistry*, 1987, **26**(4): 1107—1111.
- [6] Seto T, Tanaka T, Tanaka O. *et al.* β -glucosyl esters of 19 α -hydroxyursolic acid derivatives in leaves of *Rubus* species. *Phytochemistry*, 1984, **23**(12): 2829—2934.
- [7] Bombardelli E, Bonati A, Gabetta B, *et al.* Triterpenoids of *Terminalia sericea*. *Phytochemistry*, 1974, **13**: 2259.
- [8] Markham K R, Ternal B, Stanley R, *et al.* Carbon-13 NMR studies of flavonoids III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron*, 1978, **34**: 1389—1397.
- [9] Zhou X H, Kasai R, Ohtai K, *et al.* Oleanane and ursane glucosides from *Rubus* species. *Phytochemistry*, 1992, **31**(10): 3642—3644.
- [10] Kimata H, Nakashima T, Kokubun S, *et al.* Saponins of pericarps of *Sapindus mukurossi* Gaerth. and solbiulization of monodesmosides by bisdesmosides. *Chem Pharm Bull*, 1983, **31**: 1998—2005.